NEUROSCIENCE

It takes two to *trans*-Tango

***Trans*-Tango allows trans-synaptic mapping of presynaptic and postsynaptic partners in *Drosophila*.**

Mapping circuits in the brain is a first step towards understanding brain function. Viruses are commonly used as trans-synaptic tracers in rodents, but these tools are not necessarily restricted to single synapses, and they are most efficient in the retrograde direction. To address these limitations, Gilad Barnea from Brown University and his team wanted to develop an efficient anterograde tracer that was genetically encoded. But he quickly realized that the development of these tools would be tedious in mice. "This is why we started to work in flies," says Barnea.

The tool the researchers developed is based on the Tango system for studying signaling, which Barnea published as part of his postdoctoral work with Richard Axel at Columbia University. In the Tango system, a ligand activates a receptor, which triggers proteolytic release of a transcription factor from the plasma membrane and the expression of a target reporter gene.

To adapt the Tango system to circuit mapping, "I decided to develop a signaling pathway that will be expressed everywhere in the animal and will be activated by the population of neurons that we wish to study," explains Barnea. The components of the *trans*-Tango system are similar to previous iterations of the Tango system and include a ligand, a G-protein-coupled receptor that is fused to a transcription factor via a TEV cleavage site, a TEV protease fused to arrestin, and a reporter gene.

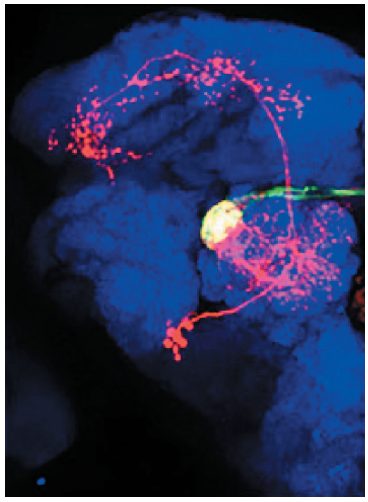
In contrast to previous Tango iterations, the ligand is attached to Neurexin1 domains, thus tethering it to the presynaptic membrane and preventing diffusion from the synapse. The ligand is only expressed in the neurons of interest ('starter' neurons), while the receptor and other downstream components are expressed throughout the nervous system, so that the reporter is only activated in cells that are postsynaptic to the starter neurons.

Barnea and his team chose to use the heterologous human glucagon receptor and a modified version of the glucagon peptide. Barnea says that this was a very good choice for them: "What was really surprising to me…was the signal to noise that was way above my hopes and expectations."

The researchers validated the *trans*-Tango tool in the *Drosophila* olfactory system, for which the circuitry is well studied, and they observed the expected connectivity between olfactory receptor neurons and local interneurons as well as projection neurons. The researchers also expressed the ligand in subsets of olfactory neurons and combined the *trans*-Tango tool with tools to achieve mosaic expression. This resulted in sparsely labeled postsynaptic neurons, which allowed Barnea and his team to examine the circuitry in more detail and to further validate the system.

Barnea and his team then turned to the fly gustatory system, which is less studied than the olfactory system. They revealed that gustatory receptor neurons involved in sweet perception connect to a large number of second-order neurons in both the central brain and in the ventral nerve cord. Again, the team refined their mapping strategy by combining *trans*-Tango with sparse labeling techniques in order to more precisely analyze and describe the connectivity in the gustatory system.

The tool should be useful beyond the sensory circuitries that the researchers have mapped. "There is no reason why it wouldn't work," says Barnea, although he concedes that "it will take some tweaking in every system, in every circuit." In fact, Barnea has already distributed the *trans*-Tango system to more than 50 labs that work on highly diverse questions in *Drosophila* neuroscience. The high demand reflects the fact that alternative tools for circuit mapping in *Drosophila* either have high background activity or are difficult to use.



Circuit tracing in *Drosophila* with starter neurons in yellow–green and target neurons in red. Image reprinted with permission from Talay *et al.* (Elsevier).

In the meantime, Barnea is refining the *trans*-Tango system to adapt it for functional studies involving, for example, optogenetic stimulation. In such studies, it would be desirable to restrict expression of the optogenetic tool to target neurons only and to avoid expression in starter neurons. This is currently not possible, as there is the possibility of 'self-activation' through activation of the *trans*-Tango receptor expressed in the starter neurons. Furthermore, Barnea has started collaborations to establish *trans*-Tango in other model organisms. He is also working on establishing a mouse version, which brings him back to his roots in mouse olfaction.

Nina Vogt

RESEARCH PAPERS

Talay, M. *et al.* Transsynaptic mapping of second-order taste neurons in flies by *trans*-Tango. *Neuron* **96**, 783–795.e4 (2017).